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ANALYSIS OF DPX-F6025 IN SOYBEANS BY LIQUID CHROMATOGRAPHY

By

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This study was conducted according to applicable good laboratory practices and meets all requirements to the best of our knowledge.

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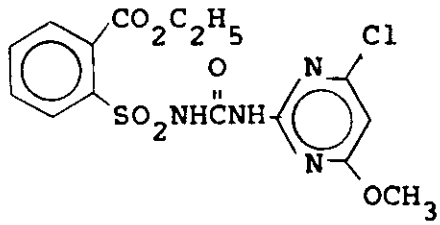
Introduction

DPX-F6025 is a herbicide developed by the Du Pont Company for the control of weeds in soybeans. An analytical method based on the use of a liquid chromatograph and a photoconductivity detector coupled with extraction, cleanup, and isolation procedures is described for its determination in soybeans. The method provides a means of determining DPX-F6025 in soybeans at levels as low as 0.01 ppm based upon a 25 gram sample.

Recoveries above 90 percent have been obtained from the detection limit of 0.01 ppm up to a fortification level of 0.04 ppm.

The structure of DPX-F6025 is shown below.



DPX-F6025

EXPERIMENTAL SECTION

Apparatus and Reagents

Liquid Chromatograph - Du Pont Model 850 equipped with controller (PN 861306-900), pump (PN 861006-000) and column oven (PN 851100-901). E. I. Du Pont Analytical Instrument Division, Wilmington, Delaware 19898.

Detector - Tracor Model 965 Photoconductivity detector Tracor Instruments, Austin, Texas.

HPLC Column - Waters™ μPorasil™ (3.9 mm x 30 cm) Cat. No. 27477 Millipore Waters Chromatography Division, Milford, Massachusetts 01757.

Recorder - Perkin Elmer R-100 1 millivolt - Perkin and Elmer Corporation, Norwalk, Connecticut.

Nitrogen Evaporator - N-EVAP® Model 111 Organomation Associates, Inc., Northborough, Massachusetts 01532.

Rotary Evaporator - Rotavapor R110 with thermo lift water bath (09-548-151C) Fisher Scientific, Pittsburgh, Pennsylvania 15238.

Silica Bond Elut® Cartridges - No. 601303 - Analytichem International, Inc., Harbor City, California 90710.

Bond Elut® Adaptor - No. 636001 - Analytichem International, Inc., Harbor City, California 90710.

Homogenizer - Tekmar SKT Tissumizer Model SDT-1810 with a Model SDT-182EN shaft and generator. Tekmar® Company, Cincinnati, Ohio.

Waring Blender - Model 7012 G, VWR Scientific, Philadelphia, Pennsylvania 19101.

Centrifuge Tubes - 15 mL No. 05-538-35B, Fisher Scientific. Pittsburgh, Pennsylvania.

Centrifuge Bottles - 250 mL No. 05-587A, Fisher Scientific, Pittsburgh, Pennsylvania.

Glass Adaptor - No. LG - 1840 - 100 24/40 joint, No. LG - 1200 - 120 24/40 joint, Lab Glass Inc., Vineland, New Jersey 08360.

Filter - Millex®-SR 0.5 µm - No. SLSR.025B, Millipore Corporation, Bedford, Massachusetts 01730.

Solvents - Methylene Chloride, Methanol, Hexane, Isopropanol, Cyclohexane - HPLC Grade Fisher Scientific, Pittsburgh, Pennsylvania.

DPX-F6025 Standard - E. I. Du Pont Agricultural Products Department, Wilmington, Delaware 19898

Water - Distilled, Deionized water obtained using a Milli-Q® Water Purification System, Millipore Corp. Milford, Massachusetts 01757.

Extraction Procedure

Grind approximately 100 g of soybeans in a Waring blender to a fine powder and mix thoroughly. Place 25 ±0.1 g of ground sample into a 250 mL centrifuge bottle. Add 100 mL of methylene chloride and homogenize the mixture for 2 minutes at maximum speed using the Tekmar® homogenizer. Filter this slurry through a Buchner funnel containing a No. 42 filter paper into a 500 mL round bottom flask. The Buchner funnel is attached to the 500 mL round bottom flask by means of the glass adaptor with the side arm attached to a vacuum source. The matrix is removed from the funnel and returned to the centrifuge bottle. Add an additional 100 mL of methylene chloride to the centrifuge bottle and swirl to mix the contents. Filter this mixture through the Buchner funnel into the 500 mL round bottom flask. Disconnect the 500 mL round bottom flask from the Buchner funnel and add 100 mL of deionized water to the solution in the round bottom flask. This flask is then attached to a rotary-evaporator and placed in a water bath at 50°C and the methylene chloride is removed under vacuum. The remaining aqueous solution, after

the methylene chloride is removed, is quantitatively transferred to a 250 mL separatory funnel. The round bottom flask is washed with an additional 25 mL of water and the wash is added to the separatory funnel. The aqueous phase is washed with three separate portions of hexane 100 mL. The first wash should be gentle shaking (approximately 1 minute) to avoid serious emulsions. The aqueous layer (lower layer) is drained into a second 250 mL separatory funnel and the hexane discarded. The second and third 100 mL's of hexane are added and shaken for 1 minute each time. The solution is allowed to stand to obtain clear separation of the two phases. The aqueous layer (lower layer) is drained back into the first 250 mL separatory funnel and the hexane discarded. Extract the aqueous phase 3 times with 50 mL of methylene chloride. Each extraction is shaken for 1 minute and layers allowed to separate. Drain the methylene chloride (lower layer), after each extraction, through 5 g sodium sulfate and cotton* contained in a 7.5 cm glass funnel into a 250 mL round bottom flask. Attach the 250 mL round bottom flask to the roto-evaporator and water bath held at 50°C. Evaporate the methylene chloride to approximately 5 mL under vacuum.

Clean-Up Procedure

Connect two Bond Elut® Sil cartridges in series using the Bond Elut® adaptor. A 15 mL glass centrifuge tube is placed in a 250 mL filter flask. The flask is attached to a vacuum source at the side arm. A 4 inch, 16

* Use a good quality cotton, such as "Red Cross" brand.

gauge leur nut stainless steel needle is inserted through a No. 6 rubber stopper and the stopper is placed on the filter flask with the needle extending into the centrifuge tube. The Bond Elut® Cartridges are fitted to the leur hub of the needle. Wash the column with 5 mL of methylene chloride to condition the column. Add the 5 mL sample from the 500 mL round bottom flask to the column using a Pasteur pipette and rinse the 500 mL flask with 3-4 mL of methylene chloride. Add the rinse to the column. Allow the rinse to pass through the columns. A small amount of vacuum may be necessary to maintain a rate of approximately 0.5 mL/min. Discard the methylene chloride. Elute the DPX-F6025 off of the column with 10 mL of a mixture containing 85 parts-by-volume of cyclohexane, 10 isopropanol and 5 of methanol, collecting the column eluent in a second 15 mL centrifuge tube. Evaporate this solution to dryness with a gentle stream of nitrogen using the nitrogen evaporator at a temperature of 50°C. The sample can be stored dry in a refrigerator, for several days, until it can be analyzed. At the time of analysis, dissolve the sample in mobile phase (see chromatography section) and dilute to a final volume of 1 mL. Filter the entire sample through the Millex®-SR 0.5 filter unit mounted on a 1 mL hypodermic syringe into a small vial prior to analysis. The filter is discarded after each use.

Liquid Chromatography

The photoconductivity detector (Tracor Model 965) must be used at a sensitivity of 1 x 50 to achieve the desired lower

detection level, therefore, it is essential that the chromatographic system provides good temperature control of the column and reasonably pulse-free delivery of mobile phase to minimize base line fluctuations.

The photoconductivity detector must be used for this analysis to obtain adequate sensitivity and selectivity. The mercury lamp is used in the detector since it provides much greater sensitivity than the zinc lamp. The detector, including the lamp, is left on at all times to insure greater stability. The flow of the mobile phase through the reference and analytical loops is balanced to within ±5%. This is accomplished by installing a metering valve in the solvent line which exits from the reference compartment of the conductivity cell. The "T" that brings the two solvent lines from the conductivity cell back together is eliminated from the instrument. Also, the ion exchange resin tube is not needed to purify the mobile phase and might actually introduce unwanted materials into the system if it is not removed.

The mobile phase consists of a mixture of 750 parts-by-volume of hexane, 125 of isopropanol, 125 of methanol, 2 of glacial acetic acid and 1 of distilled water.

The column is a Waters™ μPorasil™ (3.9 mm x 30 cm) controlled at 35°C. A new column has to be conditioned by pumping a conditioning solution (10 parts by volume of 2-propanol, 10 of methanol, 5 of glacial acetic acid, and 1 of water) through it for several hours at 1 mL/min. This treatment is also used to clean columns which have started to

lose their efficiency because of contamination from samples. A contaminated column is characterized by broad peaks that tail very badly and by shifting retention times. This conditioning solvent must be thoroughly flushed from the column with the mobile phase. An hour of flushing at 1.0 mL/min is usually sufficient.

A sample valve, with a 20 µL sample loop, is used for manual injection of standards and samples, to minimize contamination of the HPLC column and broadening of the chromatographic peaks.

During normal operation mobile phase is pumped through the column at 1.0 mL/min. At this flow rate, DPX-F6025 elutes from the column in 5-6 minutes, depending on the extent of the column deactivation.

Standardization

A standard stock solution of DPX-F6025 is prepared by weighing out 10.0 mg, dissolving it in methylene chloride, and diluting to 100 mL in a volumetric flask. This solution is quite stable and can be stored for many months if it is stored in a refrigerator. However, long-term storage and repeated use of this solution may result in the evaporation of some methylene chloride, thereby increasing the concentration of the DPX-F6025.

The working standards used for liquid chromatography as well as for the spiking of recovery samples, are prepared by pipetting 1.0 mL of the stock solution into a clean, dry,

100-mL volumetric flask, and diluting to volume with methylene chloride. Standards with concentrations of 0.25, 0.50, 1.0, 1.5, and 2.0 µg/mL are prepared from the 1.0 µg/mL standard by appropriate dilution with methylene chloride. The set of standards prepared in methylene chloride are replaced with a fresh set every month. Over this time period, based on detector response these standards were stable. All standards are stored in a refrigerator when not in use.

The detector output is linear over this particular range of DPX-F6025. The minimum detectable quantity of DPX-F6025 put through the chromatography column was 0.25 µg/mL and this amount produced a peak 23 mm in height when the detector is operated at a sensitivity of (1 x 50) and a 1-MV recorder with a chart width of 25 cm as the readout device. In order to obtain the required detection level of 0.01 ppm, a similar level of response is necessary.

Calculations

The amount of DPX-F6025 in a given sample can be calculated from the following equation.

$$\text{PPM DPX-F6025} = \frac{H \times F.V.}{RF \times W}$$

(a) H is the peak height in millimeters.

(b) RF is the response factor in mm per micrograms per mL which is the slope of the calibration curve.

(c) F. V. is the total volume of the sample extract in mL.

(d) W is sample weight in grams.

Fortification Studies

At least 1 fortification should be run with each set of samples, covering the detection limit of 0.01 ppm to the highest level expected. A sample of 25 ±0.1 grams of ground soybeans is fortified with 1 mL of the appropriate concentration of standard prior to extraction. The sample is then processed in the normal way.

Discussion

The chromatograms of an DPX-F6025 standard, an extract of a soybean control and the same control fortified with 0.02 ppm DPX-F6025 are shown in Figure 1.

Recoveries on a number of soybean samples fortified over the range of 0.01 ppm to 0.04 ppm had a average recovery of 93 ±7 percent (Table 1).

CALIBRATION CURVE

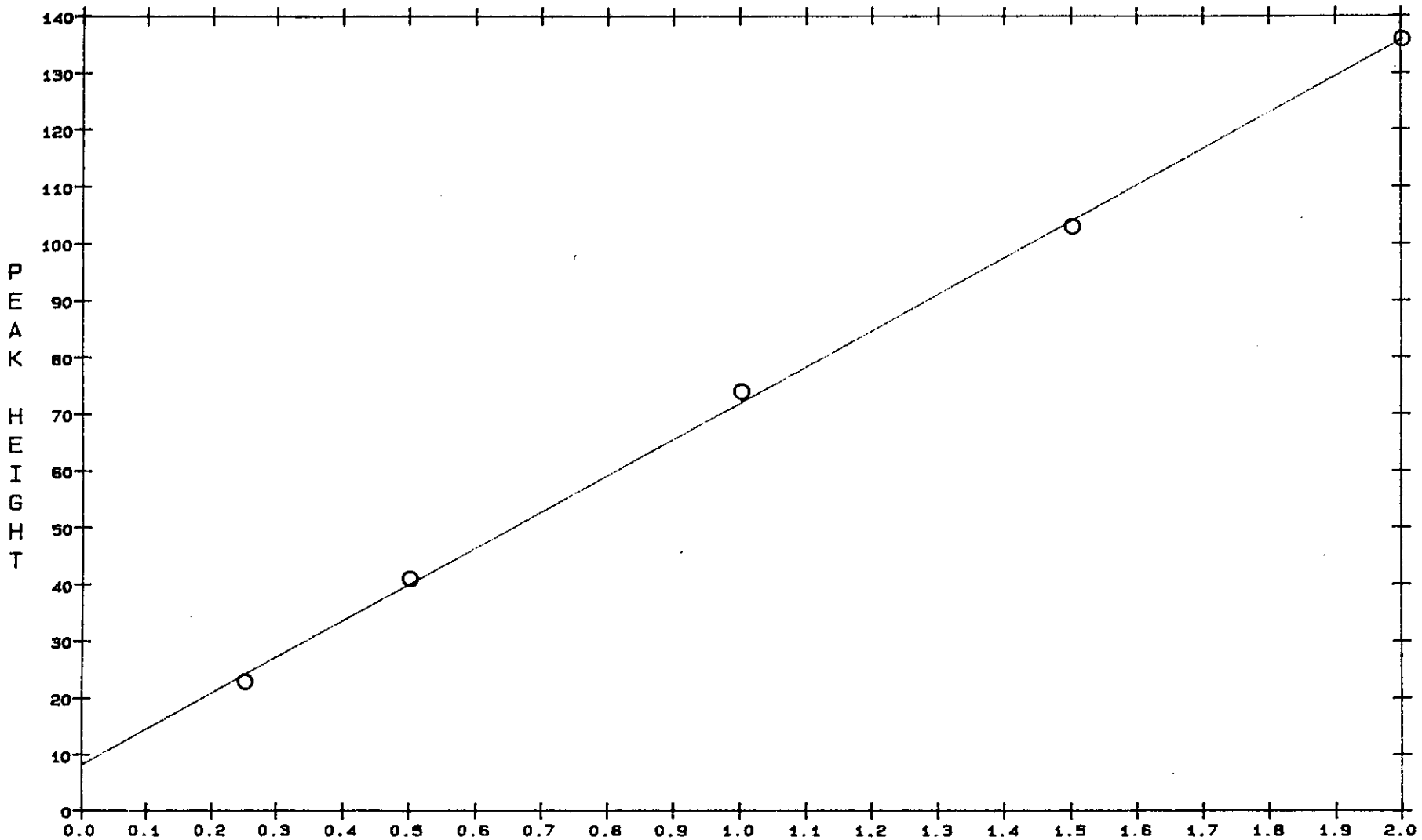


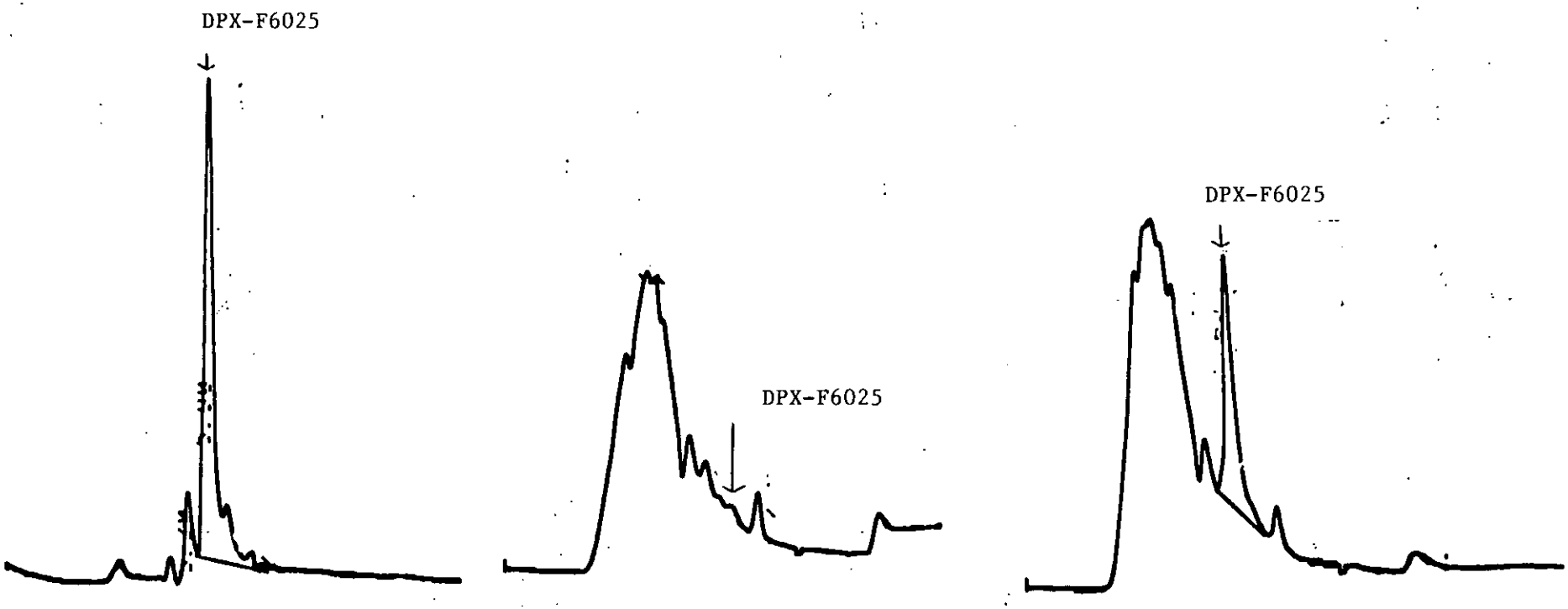
TABLE I

DPX-F6025 RECOVERIES FROM SOYBEAN

Fortification (ppm)	Number	Average % Recovery	ISD, %
Control	20	ND	---
0.01	15	95	5
0.02	15	90	10
0.04	10	91	5
		Average = 93	±7

FIGURE I

Representative Chromatograms From Soybean
Fortified With DPX-F6025



0.04 ppm Standard

Control Soybean

0.02 ppm Control
Soybean Fortification